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TREATABILITY STUDIES OF TRIBUTYLTIN IN ACTIVATED SLUDGE

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Organotin-contaminated wastewater is generated during dry dock and ship cleaning activities. We conducted a study to determine the effectiveness of activated sludge in treating contaminated wastewater. Bench-scale activated sludge reactors and state-of-the-art analytical techniques were used to determine the fate of tributyltin and its degradation products. We found that tributyltin degraded to dibutyltin and monobutyltin in activated sludge at the bench scale. Tributyltin also degrades under anaerobic conditions, and its degradation products are not readily volatilized and tend to sorb onto sludge solids.

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ABBREVIATIONS

ATA	Anaerobic toxicity assay
atm/mol	Atmospheres per mole
BMP	Biological methane potential
CMT	Carboxymethyl tartronate
COD	Chemical oxygen demand
CSTR	Continuous-stir tank reactors
DBT	Dibutyltin dichloride
GC-FPD	Gas chromatography-flame photometric detection
L/day	Liters per day
L/min	Liters per minute
MBT	Monobutyltin trichloride
m^3	Cubic meters
µg/day	Micrograms per day
µg/L	Micrograms per liter
mg/L	Milligrams per liter
mL	Milliliters
mL/min	Milliliters per minute
ng/L	Nanograms per liter
pg/L	Picograms per liter
ppb	Parts per billion
RREL	Risk Reduction Engineering Laboratory
TBT	Tributyltin Chloride
TKN	Total Kjeldahl number
TOC	Total organic carbon
WERL	Water Engineering Research Laboratory

ABSTRACT

Organotin-contaminated wastewater is generated during dry dock and ship cleaning activities. We conducted a study to determine the effectiveness of activated sludge in treating contaminated wastewater. Bench-scale activated sludge reactors and state-of-the-art analytical techniques were used to determine the fate of tributyltin and its degradation products. We found that tributyltin degraded to dibutyltin and monobutyltin in activated sludge at the bench scale. Tributyltin also degrades under anaerobic conditions, and its degradation products are not readily volatilized and tend to sorb onto sludge solids.

ADMINISTRATIVE INFORMATION

This project was conducted for the David Taylor Research Center under the Navy's Shipboard Pollution Abatement Exploratory Development Program, Task Area YM3E80004, Program Element 62233N, and Center Work Unit 2830-102. The contracting officer's technical representatives were Linda Copeland and Kathy Schultz.

INTRODUCTION

The success of tributyltin (TBT) compounds in inhibiting the growth of marine organisms has led to their use as pesticides in marine antifouling paints. During the life cycle of the paints, dry dock and ship cleaning activities may produce wastewater streams contaminated with tributyltin compounds.

BACKGROUND

Options available to treat organotin wastewater include activated carbon adsorption, incineration, and discharge to a sewage treatment plant.

Disposal of contaminated carbon in landfills will become more difficult and expensive as existing sites reach their capacity and regulatory restrictions increase. Incineration is expensive also; therefore, discharge to an existing sewage treatment plant is the easiest and most economical method of disposal.

Activated sludge treatment is the most commonly used process at municipal sewage treatment plants. The process uses naturally occurring microorganisms to degrade organic matter and remove it from wastewater streams. Municipal wastewater treatment plants are a good choice to treat organotin wastes because: (1) substantial dilution is available, which minimizes the toxic impacts of TBT compounds; (2) a diverse biomass is present, which enhances the potential for degradation of toxic compounds; (3) nutrients and cometabolites required for successful operation of biological processes are present; and (4) such treatment plants are readily available throughout the country. It is important to know the speed and extent of degradation that can be achieved by this method.

A bioreactor specifically developed to treat organotin-contaminated wastewater is an alternative to treat organotin waste. This method allows pretreatment of the wastewater at an industrial facility before it is discharged to a municipal plant. Development of a suitable bioreactor requires that researchers identify and improve microorganisms that can degrade organotin compounds.

OBJECTIVE

Our objective was to determine the fate of organotin compounds in activated sludge systems.

APPROACH

Our approach to achieve the objective was to conduct an extensive literature search in the areas of biodegradation of organic pollutants by activated sludge, biodegradation of organotin compounds, and analytical methods for organotin analysis. Also, we planned to conduct laboratory studies to determine the rate and extent of degradation of tributyltin in the activated sludge system.

The Risk Reduction Engineering Laboratory (RREL) in Cincinnati, Ohio (formerly the Water Engineering Research Laboratory) is the Environmental Protection Agency's (EPA) national center for research on wastewater treatment. The RREL developed a research plan to determine the effectiveness of activated sludge to treat organotin; this plan was used to accomplish the objective.

LITERATURE SEARCH

BIODEGRADATION BY ACTIVATED SLUDGE

Many studies have investigated the use of activated sludge to degrade pollutants. Barth, et al,[1] studied the biodegradation of carboxymethyl tartronate (CMT). They concluded that CMT is biodegradable by activated sludge; however, a 14-week acclimation period was necessary before the biomass developed a population capable of efficient degradation. Once acclimated, the biomass retained the capacity to degrade CMT for at least 1.6 times the sludge age in the absence of the substrate. This information could have important implications for organotin degradation by activated sludge, because the flow of contaminated water to the treatment plants is not expected to be continuous. Activated sludge treatment plants may not be suitable to treat wastewater if a long acclimation period is required and the population cannot degrade the TBT quickly.

Activated sludge is not an acceptable treatment method for all pollutants. MacNaughton, et al,[2] found that hydrazine can cause significant deterioration of the biomass in an activated sludge plant if the concentration in the influent exceeds 5 to 10 mg/L. The effluent hydrazine concentration was below detectable limits only at the lowest hydrazine concentrations tested (under 1 mg/L). Therefore, activated sludge is not recommended to treat waste hydrazine fuel. Both of the preceding studies were conducted using bench-scale activated sludge reactors, and

the results will vary depending on the substrate being treated. Similar studies were conducted by Malina and Sayigh;[3] Hunter, et al;[4] and Gaudy, et al.[5]

ORGANOTIN STUDIES

While biodegradation studies of organotin have never been conducted in activated sludge, biodegradation has been studied in water (Maguire, et al[6]), under estuarine conditions (Donard and Weber[7]), and in pure cultures of microorganisms (Barug[8]). Tributyltin is assumed to follow stepwise degradation to the dibutyl and monobutyl forms, and eventually to inorganic tin (Matthias, et al[9]). Toxicity decreases with each debutylization. Maguire[10] suspects that inorganic tin experiences methylation in the environment, but the final organic forms are uncertain.

Two studies were conducted to determine if the introduction of organotin into an activated sludge system would impair the ability of the activated sludge to treat the wastes. These studies by the Navy showed that unacclimated sludge from a domestic waste treatment plant was inhibited by organotin dosages as low as 25 µg/L. However, tests performed with activated sludge acclimated with a tributyltin oxide concentration of 1000 µg/L indicated no inhibition for dosages up to 8 mg/L. Studies also demonstrated that neither the performance nor the stability of a full-scale municipal activated sludge plant were discernibly influenced when wastewaters with organotin concentrations to 75 µg/L were treated.

ANALYTICAL METHODS

The limitations of the analytical methods used previously by the Navy did not allow determination of the degradation and ultimate fate of the organotin compounds. It is essential to know the type and location of the organotin degradation products in the activated sludge process and the kinetics of degradation to be able to

predict the success of the municipal treatment plant as an alternative. Recent advances in analytical techniques have made this possible.

New analytical techniques (Matthias, et al,[9] and Muller[11]) use simultaneous hydridization/extraction and gas chromatography-flame photometric detection (GC-FPD). The authors report detection limits in the ng/L range for 700-mL samples. Atomic absorption methods (Parks, et al[12]) have achieved a sensitivity as low as 1.0 pg/L for large samples. These methods are used routinely for samples in freshwater or salt water. Some difficulties occurred in applying these methods to sludge samples. TBT adheres very strongly to surfaces. Performance of the aforementioned analytical methods requires that TBT first be extracted from the sludge solids into an appropriate solvent; no extraction method exists.

The analytical method used in this project involved simultaneous hydridization/extraction of tin compounds with methylene chloride and GC-FPD determination. An internal standard was used to obtain accurate results.

RESEARCH WORK

The Risk Reduction Engineering Laboratory has an on-going program on toxics treatability with the following major objectives: (1) develop standard bench-scale treatability protocols to assess the fate of toxics in municipal wastewater treatment plants by the major removal mechanisms (sorption on solids, volatilization or stripping to the atmosphere, and biodegradation); (2) develop a kinetic data base on the removal of specific toxic organic compounds by the major treatment mechanisms; (3) correlate treatability data with fundamental physical/chemical properties of the compounds; and (4) develop methods or models to predict the fate of toxics during wastewater treatment.

The research program consisted of three phases. Phase I established analytical methodology to recover TBT and related compounds from various matrices. Phase II assessed abiotic mechanisms to remove TBT, and Phase III focused on the biotic mechanisms of aerobic and anaerobic biodegradation.

PROJECT OBJECTIVES

The objectives of the research effort are listed below.

1. Develop extraction procedures to recover TBT and its degradation products from sewage sludge.

2. Measure sorption of TBT and related compounds on raw, mixed-liquor, and digested solids from a municipal wastewater treatment plant.

3. Determine Henry's Law constants for TBT and its degradation products in a standard bubble column contactor.

4. Measure the concentration of TBT and its degradation products in the effluent from continuous-flow, bench-scale activated sludge reactors.

5. Determine acclimation times and kinetic constants for biodegradation of TBT; measure the inhibitory effects of TBT on the activated sludge process using an electrolytic respirometer.

6. Determine inhibition acclimation times and kinetic constants for anaerobic degradation of TBT using serum bottle tests and small continuous-flow reactors.

7. Correlate the fate of TBT in treatment with physical/chemical properties.

PHASE I, DETERMINATION OF ANALYTICAL PROCEDURES

Preliminary data was collected to develop and verify analytical methodology to recover TBT from various matrices. Recovery testing was performed at four concentrations (0.1, 1, 10, and 100 µg/L) of TBT, dibutyltin (DBT), monobutyltin (MBT), and other degradation products in distilled water, synthetic wastewater, raw

wastewater, and on sludge solids. Analyses were performed for all phases of the study using GC-FPD, as described in the literature for organotin compounds.[9, 11]

A method has been developed to extract butyltin compounds from sewage sludge. It was used to analyze TBT, DBT, and MBT concentrations in aqueous, solid, and whole samples of sewage sludge using dipropyltin dichloride as an internal standard.

The procedure uses simultaneous sodium borohydride ($NaBH_4$) derivatization/$MeCl_2$ extraction, with extra separation and clean-up steps involving sonification of the extract emulsion; a description follows.

1. After centrifugation, decant aqueous phase and fill a 100-mL flask. (Centrifugation is not necessary to analyze whole samples.)
2. Wash remaining solids with water and dilute to 100 mL.
3. Fill separatory funnels with sample volumes, spike with known amount of internal standard.
4. Add about 5 ml of sodium borohydride (in excess) and 3 mL of methylene chloride.
5. Shake for 10 minutes and let settle.
6. Drain extract emulsion; add 3 mL of $MeCl_2$.
7. Shake again for 10 minutes and let settle.
8. Drain and combine extract emulsions in 10-mL sample vials.
9. Place extract emulsions in ultrasonic bath for 3 to 5 minutes for separation.
10. Pipet out bottom solvent layer.
11. Dry the solvent by adding sodium sulphate.
12. Concentrate the samples by blowing air, if desired.
13. Analyze on GC-FPD.

The resulting extract is a green, yellow, or brownish clear liquid upon sonification and drying.

PHASE II, ABIOTIC STUDIES

Abiotic studies were conducted to determine volatility data and adsorption isotherms for butyltins.

DETERMINATION OF VOLATILITY DATA

Volatility tests were conducted in a bubble column contactor apparatus with a liquid-filled volume of 1 liter (length-to-diameter ratio of 12) and bubble-diffused nitrogen (Figure 1). These tests were conducted in duplicate in distilled water at a constant temperature of 23°C. TBT, DBT, and MBT were spiked at 0.5 mg/L and run concurrently with nitrogen. Nitrogen flow rate was adjusted to 70 mL/min (typical full-scale treatment plant flow rate is about 20 mL/min) to induce some volatization and allow calculation of Henry's law constant.

The solution was dumped immediately prior to testing; the columns were acid-washed, rinsed thoroughly, and filled with an identical 0.5 mg/L TBT, DBT, and MBT solution for 20 hr. The 20-hr pretreatment period was an attempt to eliminate glass surface adsorption effects during testing.

Tests were conducted over a 20-hr period, with samples taken at t = 0, 4, 13, and 20 hr. Samples were held at room temperature until tests were completed. Diphenyltin dichloride was used as the internal standard. Table 1 shows concentration values interpolated from normalized chromatogram peak areas on the calibration curve for one test run. Figure 2 is a plot of logarithmic concentration versus time; these slopes are used to calculate the Henry's law constants which are in the range of 2×10^{-5} m^3 atm/mol.

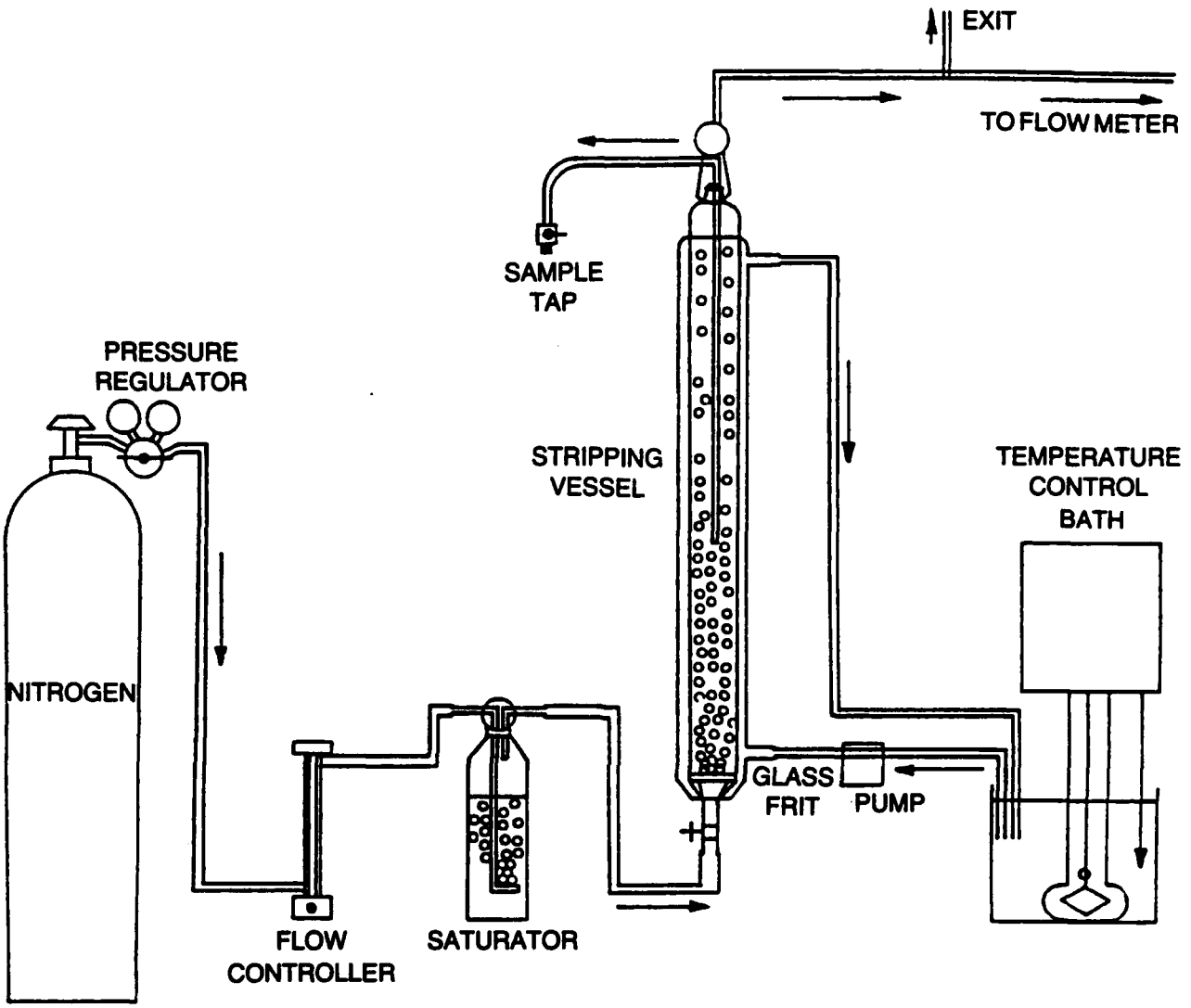


Fig. 1. Bubble column contactor apparatus.

Table 1. Volatility data for TBT, DBT, and MBT.

Time, hr	TBT, mg/L	DBT, mg/L	MBT, mg/L
0	0.50	0.52	0.51
4.25	0.47	0.49	0.49
13.25	0.47	0.48	0.49
21.75	0.45	0.47	0.47

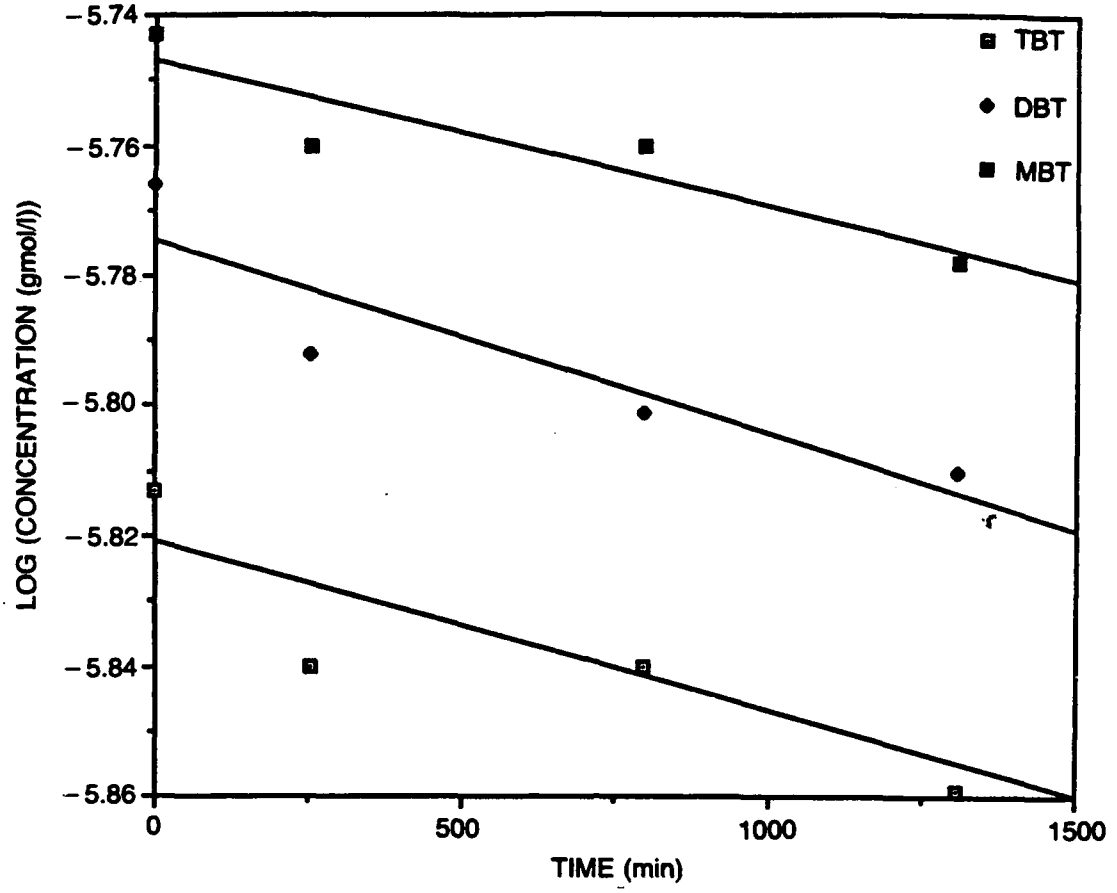


Fig. 2. Volatility plot for TBT, DBT, and MBT.

DETERMINATION OF ADSORPTION ISOTHERMS

Adsorption isotherms were developed for primary sludge, mixed-liquor suspended solids, and digested sludge from a nonindustrial treatment plant source in Fairfield, Ohio. Isotherms were obtained in triplicate in distilled water, synthetic wastewater, and primary effluent wastewater at initial concentrations of 40, 200, 400, and 1000 ppb.

Experimental Procedure

Appropriate sludges were obtained and characterized for moisture and organic content, charged at 0.2-gram dry organic solids per liter aqueous phase, and mixed vigorously for 30 minutes. Then, the sludge slurry was transferred to 500-mL flasks

and spiked independently to appropriate levels with TBT, DBT, and MBT while being stirred. Bottles were filled to 500 mL, capped, and stirred for 18 hr. Phases were separated via centrifugation, and the aqueous and solid phases were analyzed by hydridization/extraction and GC-FPD detection with diphenyltin dichloride as the internal standard. All samples were analyzed immediately after the extraction.

Data Analysis

Appendix A presents the data obtained in these runs. Table 2 gives the partition coefficients (K_p) -- the ratio of concentration in the solid and liquid phases.

Table 2. Log K_p values.

	TBT	DBT	MBT
Primary Sludge	4.06	3.40	3.31
Mixed-Liquor Suspended Solids	3.92	3.72	3.48
Digested Sludge	4.13	3.48	3.16

The values obtained can be compared with literature values of 3.87 for log K_p of TBT. Monobutyltin and dibutyltin values are not reported in the literature. These values indicate a strong tendency for TBT, DBT, and MBT to partition onto a solid phase.

Data Treatment

Initial concentration (C_0) was calculated from spike volume (μL of spike using 1 μg/L organotin in MeOH as source). Final equilibration concentration (C_e) was obtained from the calibration curve of the GC response; $X = C_o - C_e$ (the amount sorbed) was calculated. X/M versus C_e was plotted on log-log paper. M was 0.2-gram dry solids per liter of aqueous sludges (0.2 g/L) for this test. The partition

coefficient (K_p) can be calculated from the following equation, where X/M is in µg/g and C_e is in µg/L.

$$K_p = (X/M)/(C_e/1000).$$

The isotherm data can be fitted to the logarithmic form of the Freundlich equation, $\log X/M = \log K + (1/N) \log C_e$, and the Freundlich parameters obtained from the slope and intercept.

PHASE III, BIOTIC STUDIES

These studies included continuous flow bench-scale activated sludge reactors on the aerobic side and serum bottle tests for anaerobic treatment. Electrolytic respirometric studies were conducted to obtain the biokinetic constants for degradation of TBT and to determine the inhibitory characteristic of TBT on the activated sludge process.

BENCH-SCALE CONTINUOUS-FLOW REACTORS

Experimental Procedure

A series of 300-mL continuous-flow Swisher reactors (Figure 3) were charged with activated sludge from a local municipal waste treatment facility. The reactors were fed with wastewater spiked with 1.0, 12.5, 50, and 100 µg/L of TBT. Two wastewater feeds, synthetic and natural, were used. A 6-hr hydraulic retention time and 8-day sludge retention time were targeted. The process control parameters monitored throughout the acclimation and steady-state periods were mixed-liquor volatile suspended solids, chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), pH, total organic carbon (TOC), ammonia nitrate, and nitrite concentrations. Samples of liquid effluent and sludge solids were taken throughout the run. The frequency of sampling was decided after evaluation of the initial test results. A typical sampling schedule included analysis of a reactor at least once a week.

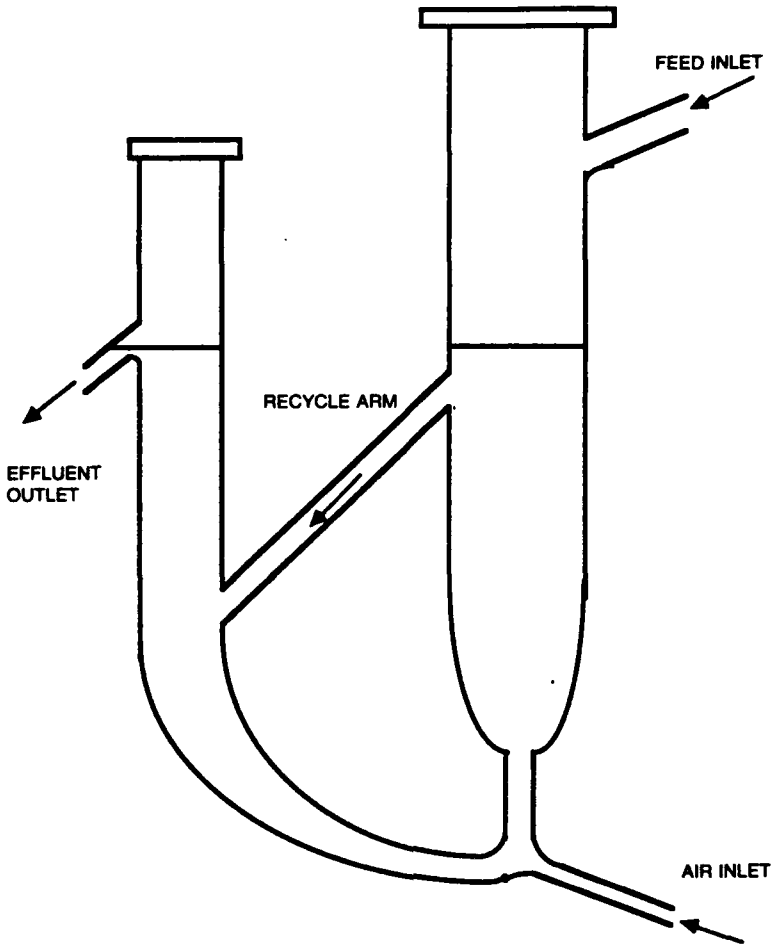


Fig. 3. Continuous biological reactor.

Data Analysis

Samples were analyzed for TBT and its degradation products to evaluate the acclimatory and steady-state performance of the continuous flow reactors. The analytical results and a mass balance were used to determine the identity and location of the degradation products in the liquid and solid phases.

Results obtained at steady state are presented in Figure 4. The results show that TBT degrades 75% to 85% in synthetic as well as natural wastewater. This is shown in Table 3, which gives the steady-state biodegradation of TBT to its degradation products (DBT and MBT).

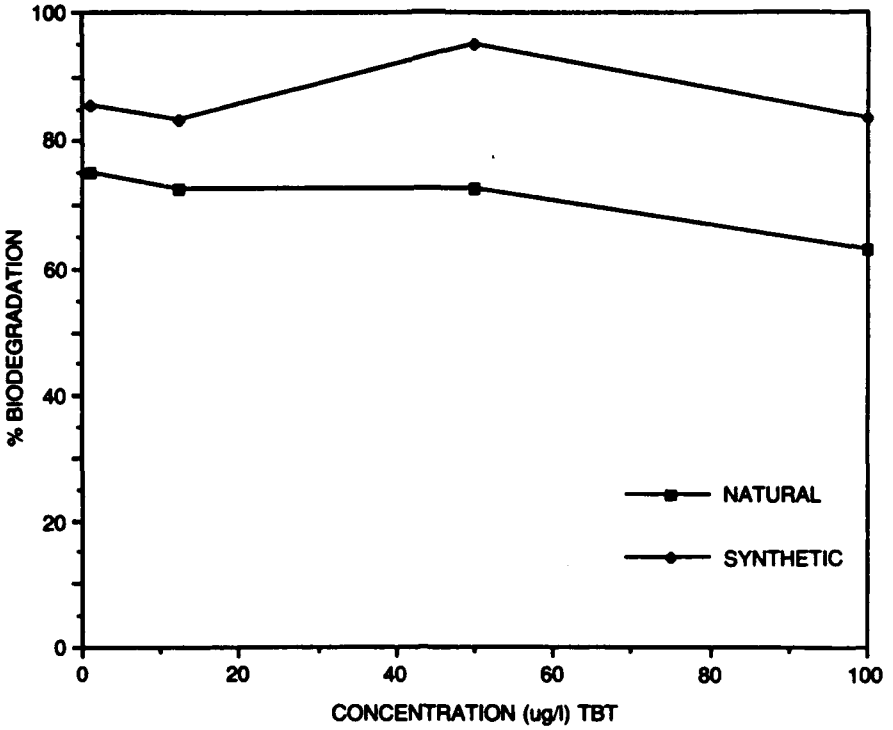


Fig. 4. Biodegradation of TBT at steady state.

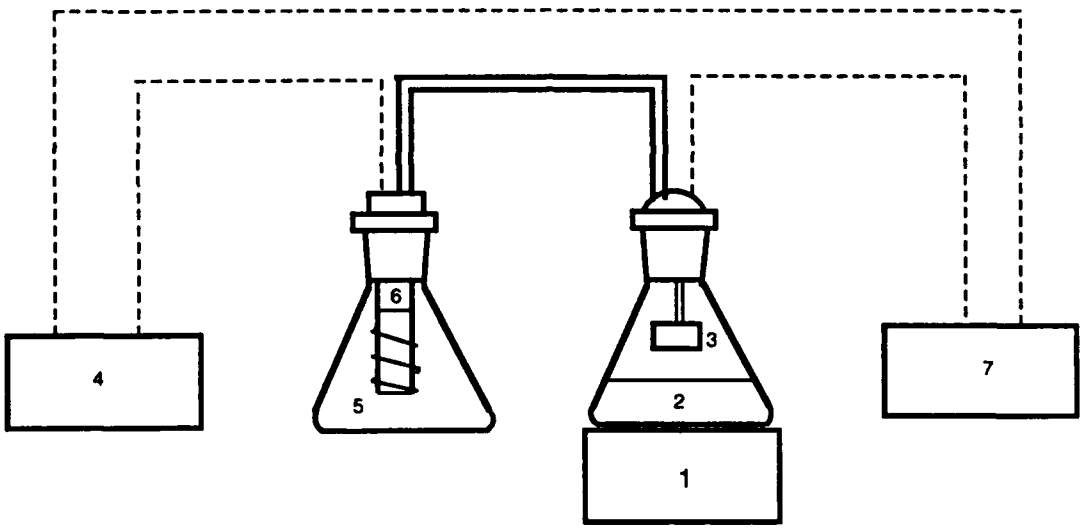
Table 3. Typical sample analysis.

Reactor No.	Concentration of TBT in Feed,* µg/day	Reactor Concentration, µg/day						TBT Degraded	
		Inlet			Outlet				
		MBT	DBT	TBT	MBT	DBT	TBT	g/day	%
Natural Feed (Recovery Factor 0.65)									
R14	1.0	0.0	0.0	1.19	0.57	0.07	0.30	0.89	75.0
R16	12.5	0.0	0.0	14.50	5.04	1.51	4.00	10.50	72.4
R18	50.0	0.0	0.0	60.00	9.23	18.50	16.60	43.40	72.3
R20	100.0	0.0	0.0	120.00	25.80	18.50	44.30	75.70	63.1
Synthetic Feed (Recovery Factor 0.42)									
R04	1.0	0.0	0.0	1.20	0.43	0.02	0.18	1.03	85.4
R06	12.5	0.0	0.0	14.61	2.53	0.00	1.65	12.14	83.0
R08	50.0	0.0	0.0	59.17	0.91	3.24	3.00	56.17	94.9
R10	100.0	0.0	7.03	120.60	6.09	7.89	20.0	100.00	83.4

*Feed to the reactors = 1.2 L/day.

INHIBITION AND BIODEGRADATION USING ELECTROLYTIC RESPIROMETRY

An automated, continuous oxygen uptake measuring Voith, Sapromat B-12 respirometer was used to study biodegradation. The instrument consists of a temperature-controlled waterbath, a recorder, and a cooling unit. The water bath contains the measuring unit shown in Figure 5. The recorder shows the digital values of each measuring unit and is connected to an IBM-AT, personal computer that collects data every 15 minutes. The cooling unit constantly recirculates water to maintain a constant temperature in the water bath. Each measuring unit consists of a reactor vessel with à CO_2 absorber mounted in a stopper, an oxygen generator, and a pressure indicator. The unit is interconnected by hoses to form an air-sealed system; hence, atmospheric pressure fluctuations do not adversely affect the results.



A. REACTION VESSEL
B. OXYGEN GENERATOR
C. PRESSURE INDICATOR

1. MAGNETIC STIRRER
2. SAMPLE (250 ml)
3. CARBON DIOXIDE ABSORBER
4. PRESSURE INDICATOR
5. ELECTROLYTE
6. ELECTRODES
7. RECORDER

Fig. 5. Electrolytic respirometry (measuring unit).

The magnetic stirrer in the sample to be analyzed provides vigorous agitation to ensure effective exchange of gases. The activity of the microorganisms in the sample creates a vacuum which is recorded by the pressure indicator and, in turn, triggers the oxygen generator. It supplies the required amount of oxygen by electrolytic dissociation of copper sulphate solution in 5% sulfuric acid. The quantity of the sample, the amperage for the electrolytic recorder, and the speed of the synchronous motor are adjusted so that with a 250-mL sample, the digital counter indicates the oxygen uptake directly in mg/L. The carbon dioxide generated is absorbed by soda lime, the nitrogen/oxygen ratio in the gas phase above the sample is maintained throughout the experiment, and there is no depletion of oxygen.

The nutrient solution used in these studies is a synthetic medium that consists of mineral salts, a trace mineral solution, and a volume of yeast extract. Aniline is used as the reference substance at a concentration of 100 mg/L.

The microbial inoculum was an activated sludge from the Little Miami wastewater treatment plant in Cincinnati, Ohio, receiving municipal wastewater. Activated sludge samples were aerated for 24 hr before use to bring them to an endogenous phase. The sludge biomass was added to the medium at a concentration of 30 mg/L. Total volume of the synthetic medium in the 500-mL capacity reactor vessels was brought to a final volume of 250 mL.

Inhibition Studies

The experimental system for inhibition studies consisted of duplicate flasks for the reference substance aniline, toxicity controls (TBT plus aniline at 100 mg/L), and inoculum control. TBT concentrations used for inhibition studies were 50, 100, 500, and 1000 μg/L. TBT stock solution was prepared in acetone, and the reactor vessels were coated with appropriate amounts of the solution to achieve

the desired concentration. Acetone was evaporated before adding the nutrient solution and make-up deionized water. The contents of the reaction vessels were stirred for 1 hr to ensure an endogenous respiration state at the initiation of oxygen uptake measurements. Then, the contents were transferred to reaction vessels coated with TBT, and aniline was added. The reaction vessels were incubated at 25°C in the dark, enclosed in the temperature-controlled waterbath, and stirred continuously throughout the run. The microbiota of the activated sludge used as an inoculum were not preacclimated to the substrates. Figure 6 contains five graphs which plot the results of the inhibition studies of TBT. The graphs indicate that TBT was not inhibitory to biodegradation in the reaction vessels.

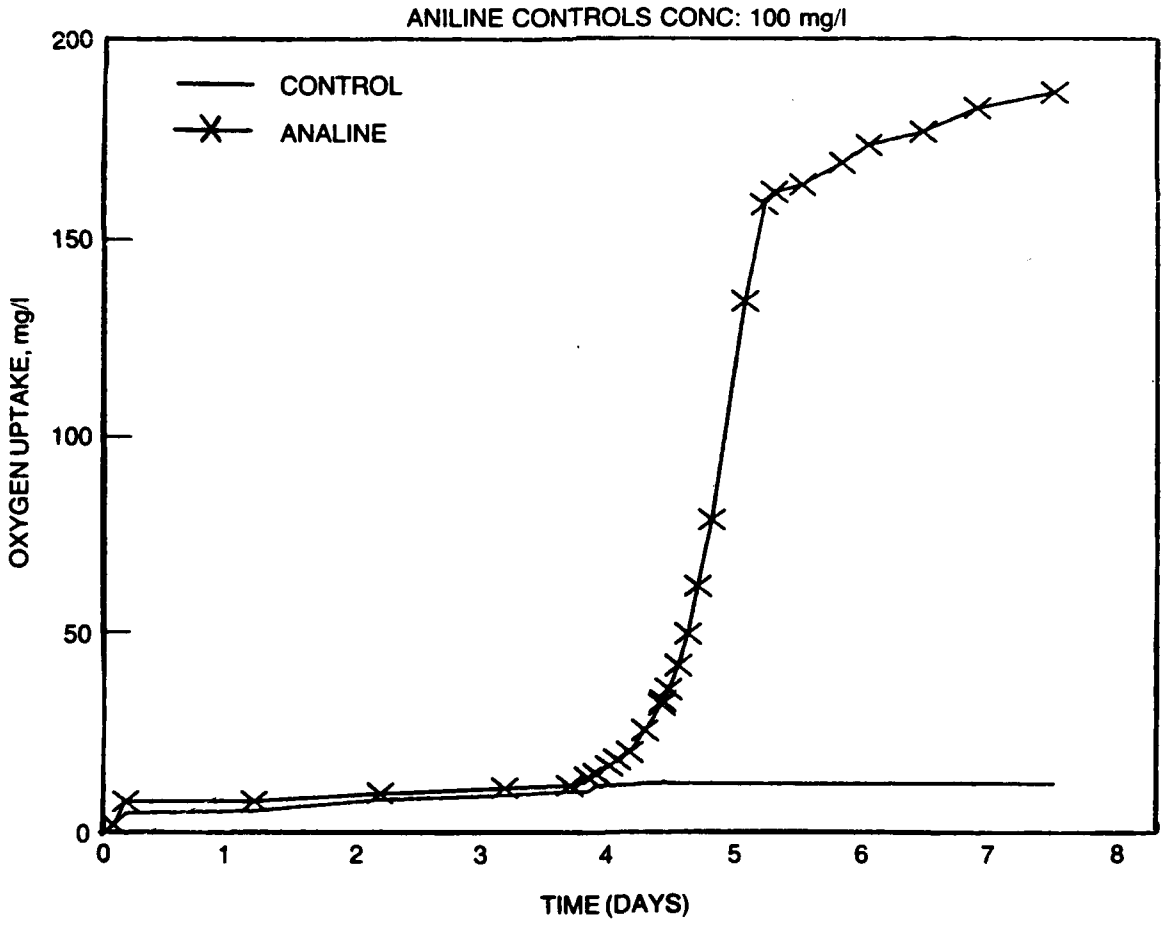
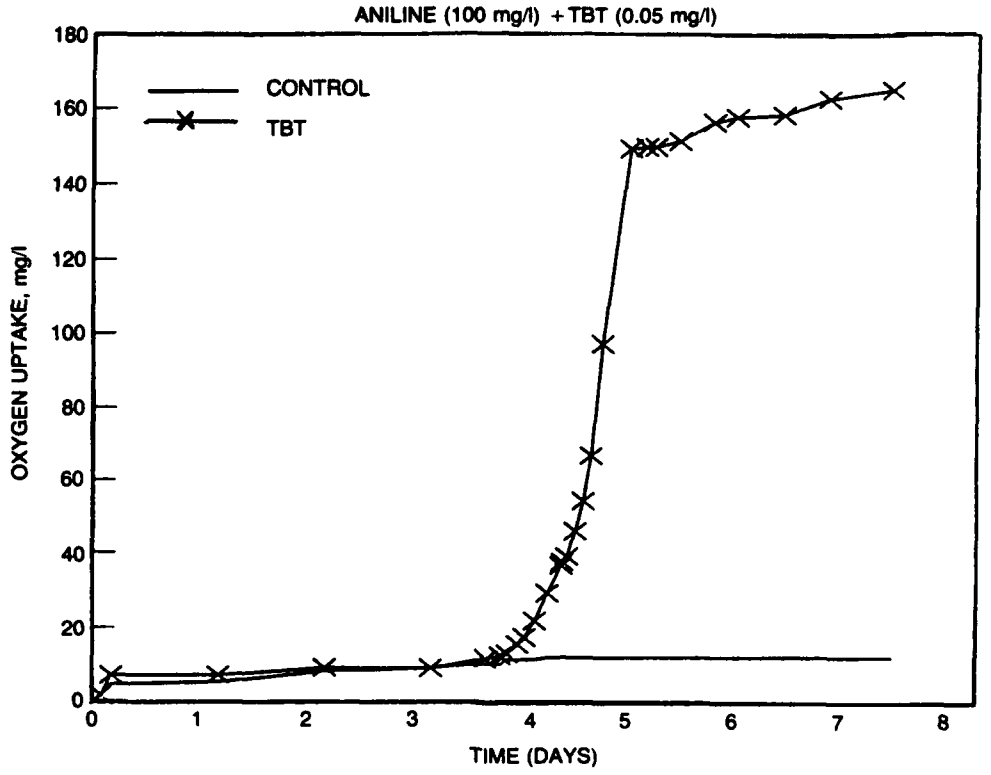
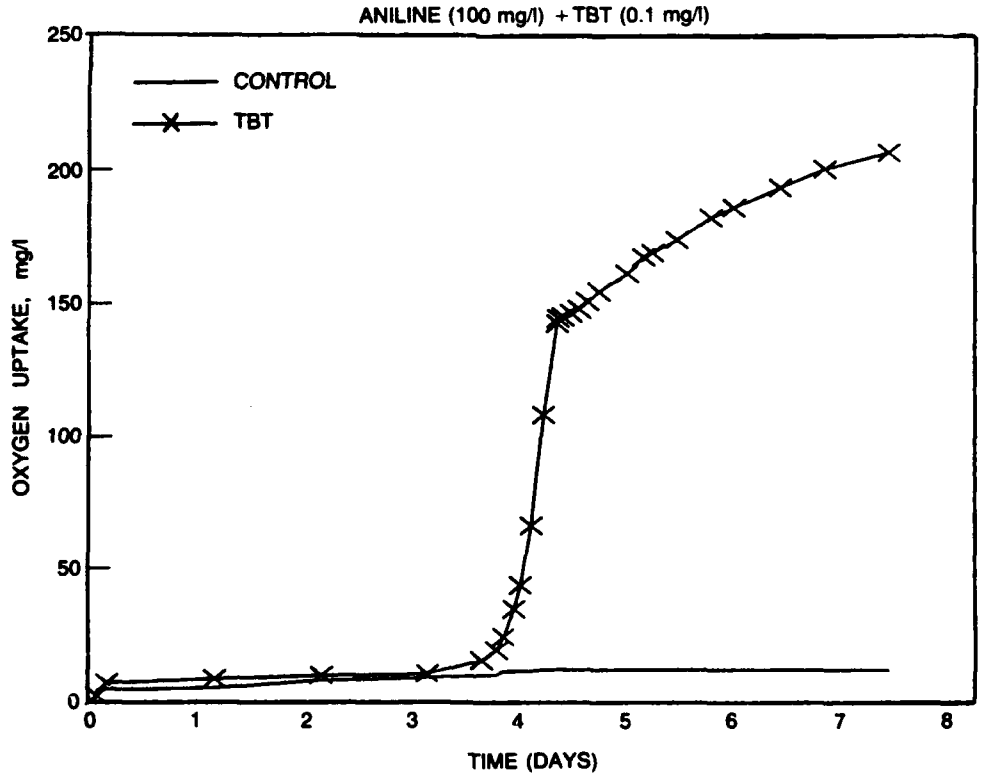


Fig. 6. Inhibition studies of tributyltin.





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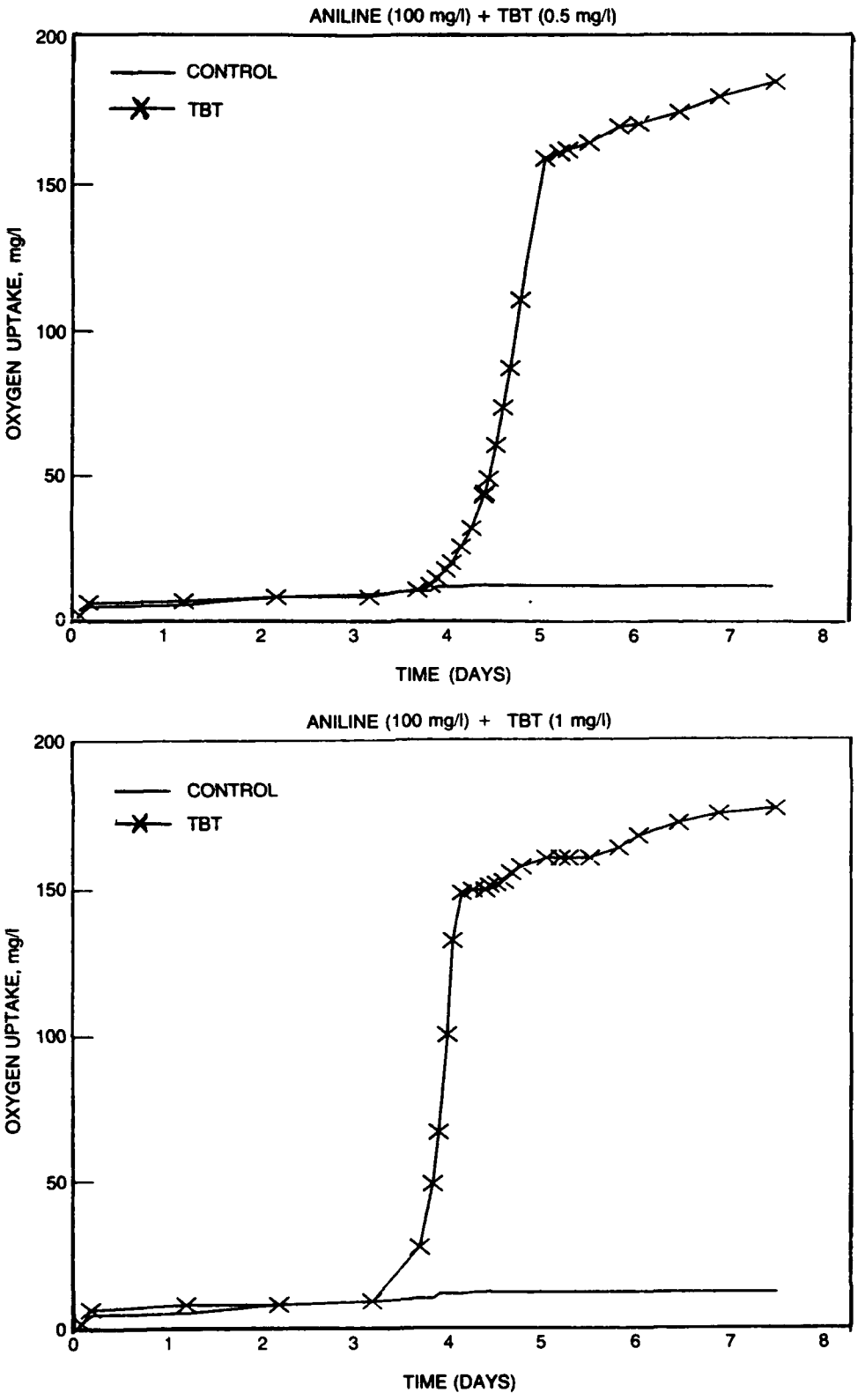

Fig. 6. (Continued)

Biodegradation Studies

Biodegradation studies employed TBT concentrations of 10, 20, and 30 mg/L. The electronic respirometry unit requires a minimum of 10 mg/L of organotin in the flasks to measure biological respiration during biodegradation studies. The experimental system consisted of duplicate flasks of the reference substance aniline at each concentration level, and single flasks for inoculum and toxicity control at each concentration level of TBT (TBT and aniline). In the experimental run, the nutrients and biomass were stirred for 1 hr to achieve an endogenous respiration state; then, the required concentrations of aniline and TBT were added directly to the reaction vessels with the aid of microsyringes. Next, the reaction vessels were incubated in the dark at 25°C, and stirred continuously throughout the run. The microbiota used for these runs were acclimated to 100 µg/L of TBT.

Anaerobic Screening Using Unacclimated Biomass

The objective of this screening was to determine the biological methane potential (BMP) of the TBT compound in anaerobic conditions. The experiments included determination of the toxicity of TBT to methanogens in the presence of acetates and propionates.

Biological methane potential and anaerobic toxicity assay (ATA) techniques were used to determine biodegradability and toxicity of TBT in anaerobic environments.[13] A description of the procedure follows.

Biological Methane Potential and Anaerobic Toxicity Assay

The BMP assay was conducted with Corning 1460, 250-mL reagent bottles; the ATA was conducted with 125-mL reagent bottles with rubber serum caps of appropriate sizes. Bottles were gassed with 30% CO_2 and 70% N_2 at a flow rate of approximately 0.5 L/min, then stoppered and equilibrated at incubation temperature prior to

introducing samples, defined media, and inocula. Defined media were prepared as suggested in Reference 13 and were stored at 4°C. The defined media contains nutrients and vitamins for mixed anaerobic cultures (Table 4). Resazurin is added to detect oxygen contamination; the addition of sodium sulfide provides a reducing environment. Table 5 gives the method used to prepare the defined media. The final assay concentrations are 12 mg/L as nitrogen, 19 mg/L as phosphorous, and 2500 mg/L as $CaCO_3$.

The defined media was equilibrated to assay temperature, inoculated, and transferred into serum bottles. Inoculation is accomplished anaerobically for the BMP assay by inserting a gas flushing needle into the neck of media flasks while 200 mL of seeding organisms are added to 1800 mL of defined media. A 20% by volume inoculum was used.

Figure 7 is a schematic diagram of the procedure for anaerobic transfer of defined media into serum bottles. Care was taken to minimize the introduction of air into the anaerobic bottles. After the bottles were capped, the bottles were zeroed with a syringe and incubated at 35°C.

Table 4. Stock solutions for preparation of defined media.

Solution	Compound	Concentration (g/L)
S1	Sample	<2 degradable COD
S2	Resazurin	1.0
S3	$(NH_4)_2\ HPO_4$	26.7
S4	$CaCl_2 \cdot 2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2 \cdot 6H_2O$	120.0
	KCl	86.7
	$MnCl_2 \cdot 4H_2O$	1.33
	$CaCl_2 \cdot 6H_2O$	2.0
	H_3BO_3	0.38
	$CuCl_2 \cdot 2H_2O$	0.18
	$Na_2MoO_4 \cdot 2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2 \cdot 4H_2O$	370.0
S6	$Na_2S \cdot 9H_2O$	500.0
S7	Biotin	0.002
	Folic acid	0.002
	Pyridoxine hydrochloride	0.01
	Riboflavin	0.005
	Thiamine	0.005
	Nicotinic acid	0.005
	Pantothemic acid	0.005
	B_{12}	0.0001
	p-aminobenzoic acid	0.005
	Thioctic acid	0.005

Table 5. Preparation of defined media.

Step	Instruction
1	Add 1 liter of deionized water to a 2-liter volumetric flask.
2	Add 1.8 mL to Solution S2, 5.4 mL to Solution S3, and 27 mL to Solution S4.
3	Add deionized water up to the 1800-mL mark.
4	Boil for 15 minutes while flushing with N_2 gas at approximately 1 L/min.
5	Cool to room temperature (continue flushing with N_2 gas).
6	Add 18 mL to Solution S7, 1.8 mL to each of Solutions S5 and S6.
7	Change gas to 30% CO_2:70% N_2 mixture and continue flushing at 1 L/min.
8	Add 8.4 grams of $NaHCO_3$ as powder.
9	Bubble 30% CO_2:70% N_2 gas mixture through porous diffuser until media pH stabilizes at approximately 7.1.
10	Carefully seal volumetric flask while minimizing introduction of air into the container.

Note: The media is prepared in a 2-liter volumetric flask marked at the 1800-mL level. It can be stored indefinitely provided oxygen is excluded. Seed organisms are added to the media prior to transfer by filling the volumetric flask to the 2-liter mark with seed (BMP only).

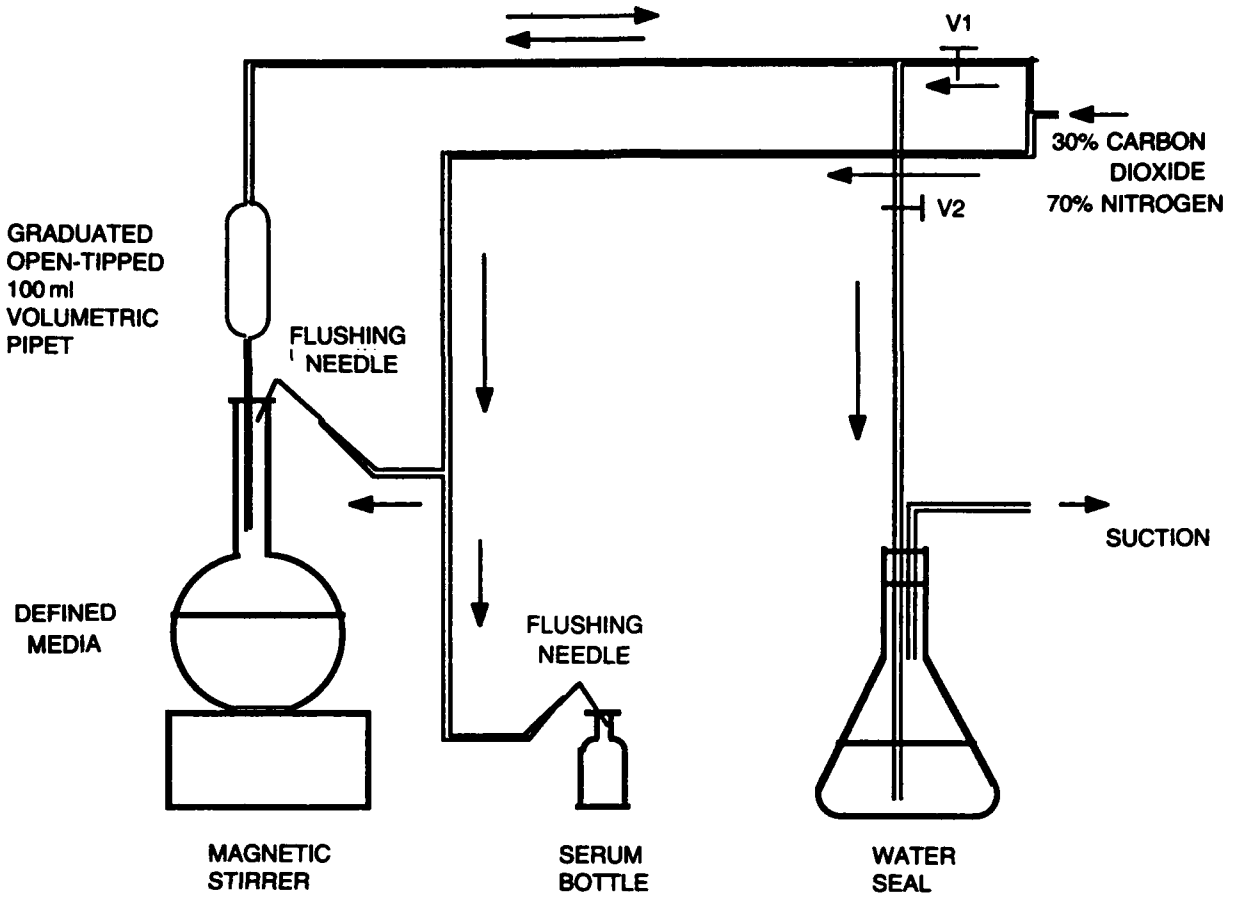

Fig. 7. Apparatus for anaerobic transfer of defined media.

Biological Methane Potential Test

Seed blanks were prepared without adding the organic substrate. Samples were anaerobically added to the bottles before the transfer of inoculated defined media, and triplicates were prepared for all samples. The concentrations chosen for these analyses were 10, 100, 500, and 1000 µg/L.

Anaerobic Toxicity Assay

In addition to the seed blanks, a "spike" containing acetate and propionate was added to each bottle. A control with only the spike was prepared also. Each bottle contained 2 mL of acetate-propionate solution, containing 75-mg acetate and 26.5-mg propionate.

Gas Measurements

Measurements of the gas produced during the first few days of these studies are critical. Gas measurements were taken with the help of a calibrated pressure transducer; gas was expelled after each measurement.

Data Analysis

Data showed that TBT degrades easily in anaerobic environments. Methane production in BMP and ATA studies showed that TBT degradation is not hampered by the presence of other biodegradable compounds.

Figures 8 and 9 show the cumulative methane gas production in ATA and BMP studies, respectively.

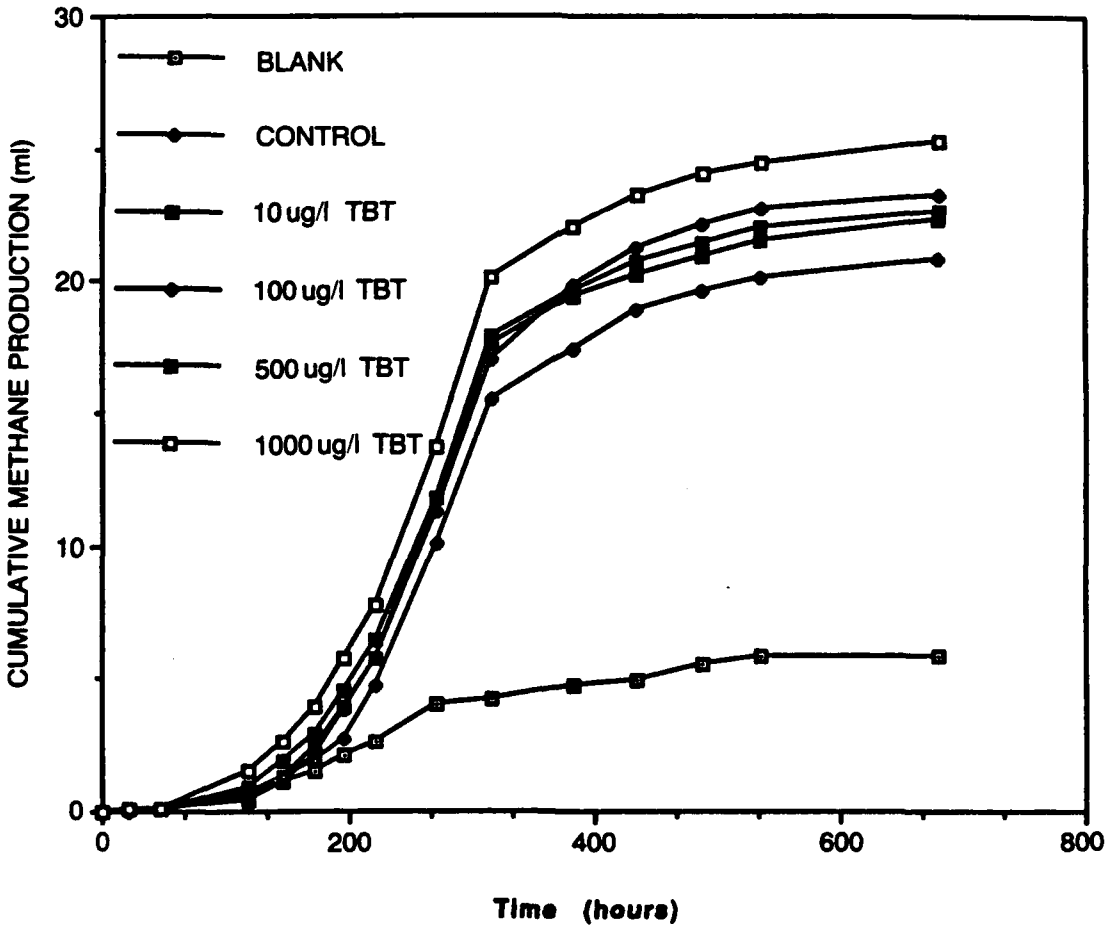

Fig. 8. Cumulative methane production in ATA studies.

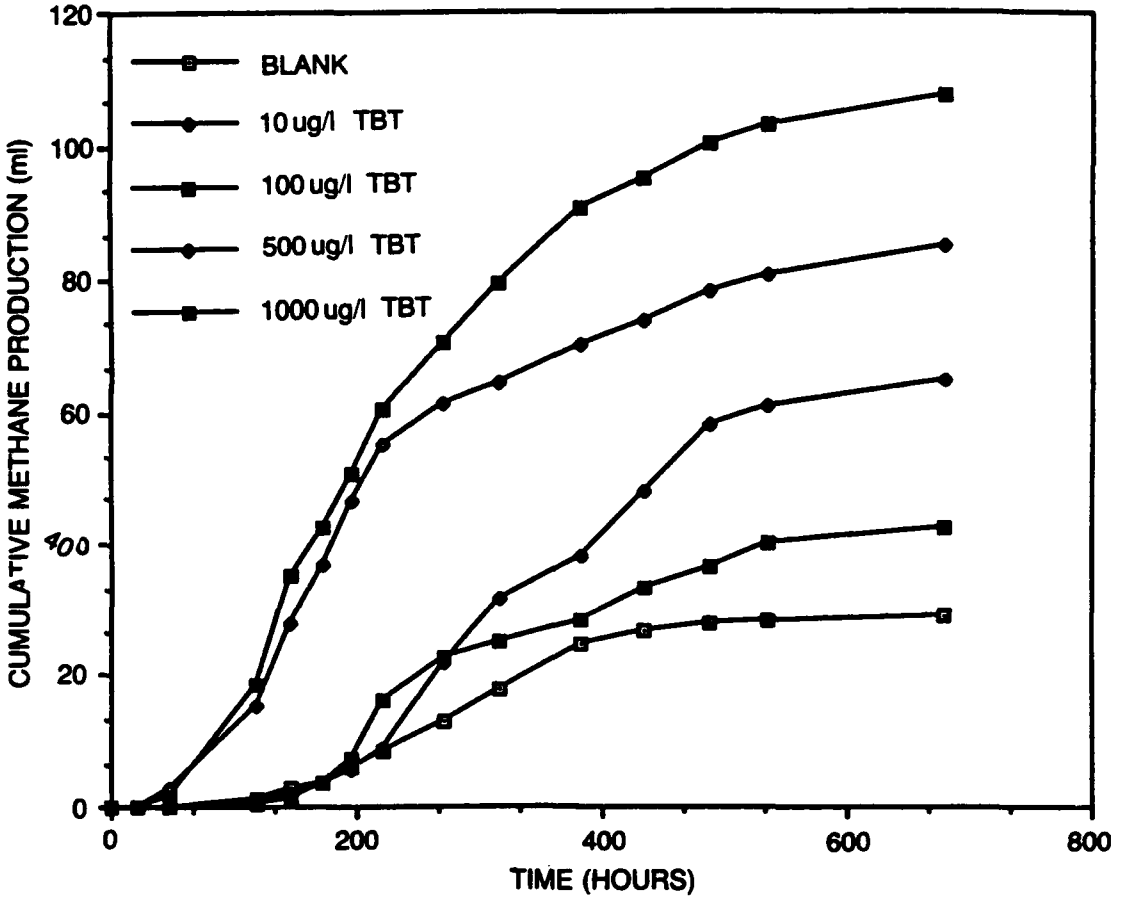

Fig. 9. Cumulative methane production in BMP studies.

RESULTS AND DISCUSSION

PHASE I

A method was developed to measure TBT concentration after review of the literature. Simultaneous hydridization with sodium borohydride and extraction into methylene chloride produced an emulsified extract. Sonification of that extract resulted in a clear solvent layer that could be dried with sodium sulfate and analyzed by GC-FPD. Dipropyltin was used as an internal standard to quantify butyltin concentrations. This allows accurate measurement of the sample injected and the concentration factor of the extracted sample (if it was concentrated prior to

injection). Whole samples were analyzed in this manner with 93% TBT, 100% DBT, and 78% MBT recovered. Split samples were prepared as above after centrifugation, with the aqueous phase separated from the solid phase. Combined recoveries of split samples were 84% TBT, 100% DBT, and 65% MBT.

PHASE II

Dibutyltin, monobutyltin, and particularly tributyltin species have a strong tendency to partition onto the solid phase. Adsorption isotherms were developed for primary sludge, mixed liquor solids, and digested sludge. Partition coefficients (K_p, concentration in the solid phase divided by concentration in the liquid phase) were measured, and the TBT values compared with literature values of 3.87 for log K_p. K_p values can be calculated:

$$K_p=(X/M)/(Ce/1000),$$

where $X = C_o - C_e$ (amount sorbed),

C_o = initial concentration,

C_e = equilibrium concentration, and

M = grams of dry solids/liter aqueous.

The isotherm is plotted as X/M versus C_e on log-log paper. The isotherm data can be fitted to the logarithmic form of the Freundlich equation, log X/M = log K + (1/N) log C_e; Freundlich parameters can be obtained from the slope and intercept. Partition coefficient values for TBT, DBT, and MBT are summarized in Table 2. These values compare well with the data reported in the literature for TBT; no data were available for DBT or MBT. The isotherms were obtained at 22°C.

Volatilization is not a primary removal mechanism for TBT, DBT, or MBT. All three organotins were spiked into distilled water in a standard bubble column

contactor; see Figure 1. Nitrogen was bubbled through the column at a flow rate of 70 mL/min to induce volatization. A typical full-scale treatment plant flow rate is 20 mL/min. Table 1 indicates no significant stripping of TBT, DBT, or MBT.

PHASE III

Bench-Scale, Continuous-Stir Tank Reactor Studies

Continuous-flow, bench-scale reactors (Figure 3) were run with an approximate 6-hr hydraulic retention time and 8-day solids retention time. They were charged with activated sludge from a local municipal waste treatment facility. The reactors were fed with two types of wastewater -- primary effluent from a waste treatment plant and synthetic wastewater. Feed and effluent streams to the reactor were sampled and analyzed for TBT and its degradation products. TBT, DBT, and MBT were found in the effluent stream; the mass balance indicated that the TBT was converted to DBT and MBT simultaneously. The percent conversion of TBT to its products was found to be independent of the feed concentration. The percentage of degradation appeared to be 10% to 15% higher in reactors fed with synthetic wastewater. The biodegradation obtained at steady state was in the range of 75% to 85% at all concentrations. Table 3 lists the values obtained.

Inhibition Studies

Studies were conducted to determine if TBT inhibits the oxygen uptake of unacclimated biomass or the degradation of the reference substance, aniline. An automated Voith Sapromat B-12 respirometer was used to measure oxygen uptake continuously from air-sealed reactors. Reactor vessels were inoculated with biomass from activated sludge and fed a nutrient solution. Aniline was used as the reference substance that the unacclimated biomass degraded, consuming oxygen in the process. Toxicity controls containing TBT at four different concentrations, and aniline, were

similarly monitored for oxygen uptake for 7 days. Concentrations of TBT up to 1000 µg/L did not inhibit the degradation of aniline, and oxygen uptake curves for aniline and toxicity controls were the same. Also, TBT was degraded 80% to 90% based on the analysis of culture samples.

Biodegradation Studies

The oxygen uptake of the reference substance aniline, toxicity controls, and TBT concentrations of 10, 20, and 30 mg/L were followed for 30 days. The oxygen uptake of TBT at all concentrations was lower than that of the inoculum control, which indicated that there was no microorganism activity. This not only suggests that there was no degradation of TBT at concentrations of 10, 20, and 30 mg/L, but also that TBT was toxic to the microorganisms. The oxygen uptake of the toxicity controls was similar to that of TBT, which indicated that it was inhibitory at these levels. The biomass used for these studies was acclimated to low concentrations of TBT (100 µg/L). The results are conclusive under the conditions and concentrations studied.

The high concentrations of TBT required for the electronic respirometry probably account for the inhibition and lack of degradation, which is contrary to results from studies performed at <100 µg/L concentrations. Other techniques are necessary to determine the biodegradation kinetics at low concentrations.

Anaerobic Toxicity Studies

Biodegradability and toxicity of TBT in anaerobic environments was determined by the biological methane potential test and the anaerobic toxicity assay. Mixed anaerobic seed cultures were added to serum bottles. Four different concentrations of TBT were used, and the production of methane was measured. Also, measurements

were made of the TBT and its degradation products. We found that TBT easily degrades in anaerobic environments and its degradation is not hampered by the presence of other biodegradable compounds.

CONCLUSIONS

1. The extraction procedure developed for the recovery of TBT from sewage sludge can be applied to other compounds.
2. TBT will degrade to DBT and MBT in an activated sludge system.
3. TBT will degrade under anaerobic conditions.
4. TBT is not readily volatilized.
5. TBT has a strong tendency to sorb onto solids.

RECOMMENDATION

We cannot estimate how fast TBT will degrade in a municipal sewage treatment plant because kinetic studies requiring techniques other than electrolytic respirometry are required. Kinetic studies allow us to determine if the degradation rate is fast enough to provide adequate treatment during typical municipal treatment plant activated sludge retention times of less than 12 hr. If the rate is not fast enough, results of the studies described in this report show that an activated sludge bioreactor could be designed with adequate retention time to provide pretreatment prior to discharging its effluent into a municipal system.

APPENDIX A

GRAPHS USED TO DETERMINE ADSORPTION ISOTHERMS

C_o	C_e	X	XM	log K_p
10	8	2	10	3.19
50	32	18	90	3.19
200	173	27	135	3.20
500	361	139	695	3.21
1000	725	274	1370	3.03
				Average log k_p = 3.16

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	8	6	9
50	29	231	37
200	185	180	153
500	370	350	363
1000	720	802	653

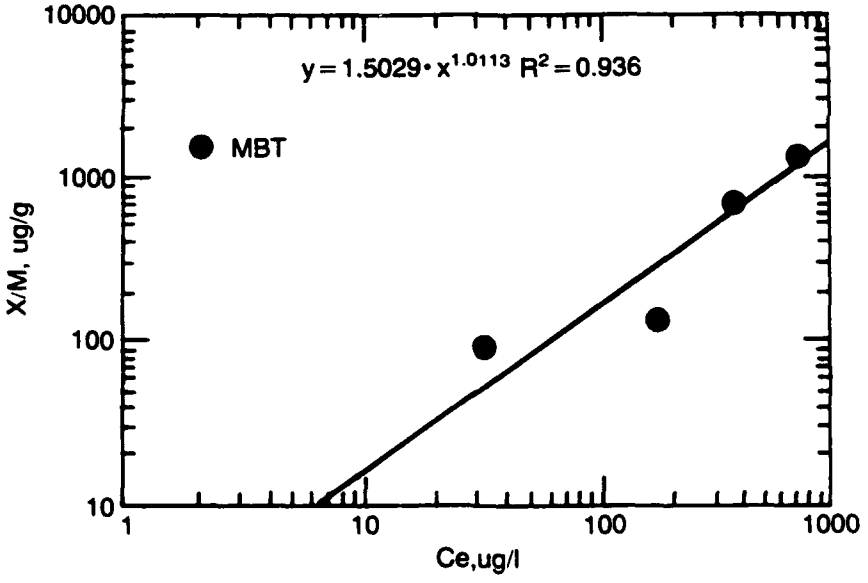

Fig. A.1. Monobutyltin in digested sludge.

C_o	C_e	X	XM	log K_p
10	6	4	20	3.65
50	33	17	85	3.54
200	77	123	615	3.49
500	320	180	900	3.39
1000	793	207	1035	3.34
				Average log k_p = 3.48

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	6	5	6
50	47	21	30
200	68	83	80
500	343	318	299
1000	810	780	790

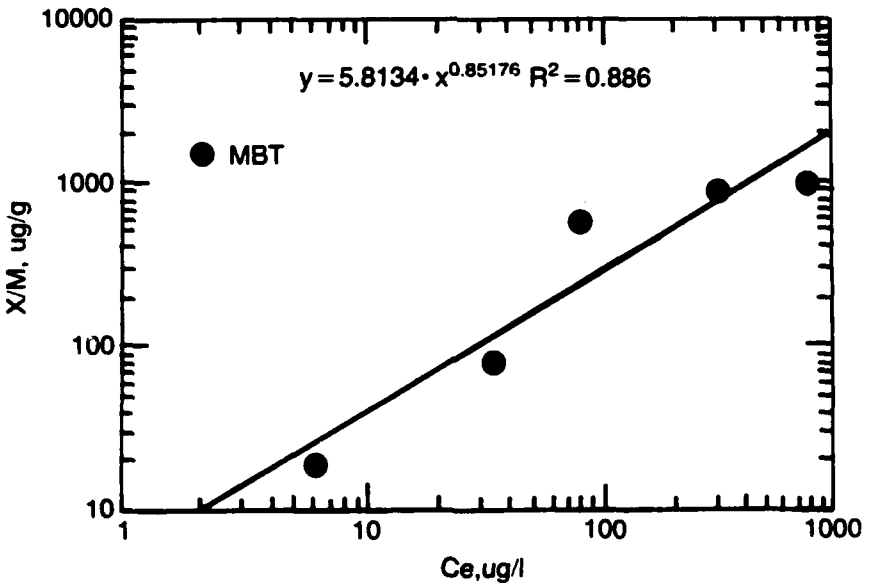

Fig. A.2. Monobutyltin in mixed sludge.

C_o	C_e	X	XM	log K_p
10	7	3	15	3.39
50	30	20	100	3.35
200	161	39	195	3.29
500	350	140	700	3.27
1000	709	291	1455	3.25
			Average log k_p =	3.31

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	6	6	8
50	27	22	42
200	180	158	146
500	401	377	304
1000	172	659	758

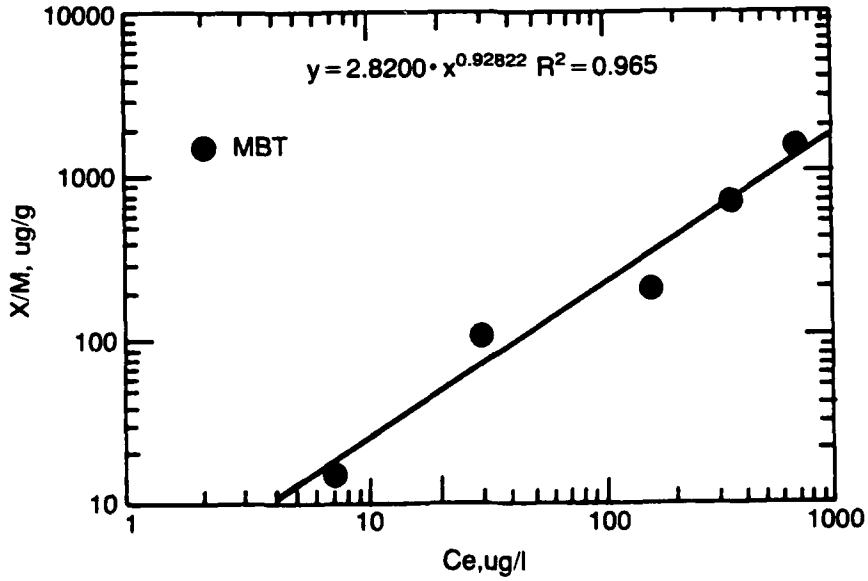

Fig. A.3. Monobutyltin in primary sludge.

C_o	C_e	X	XM	log K_p
10	5	5	25	3.63
50	29	21	105	3.54
200	160	40	200	3.45
500	325	175	875	3.21
1000	547	453	2255	3.39
				Average log k_p = 3.48

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	3	5	6
50	30	35	23
200	149	164	167
500	277	367	330
1000	513	502	525

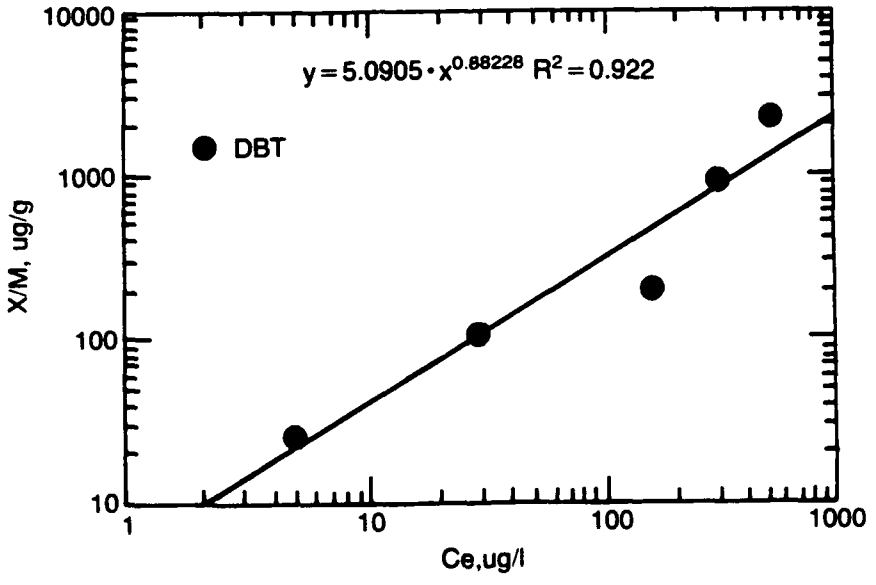

Fig. A.4. Dibutyltin in digested sludge.

C_o	C_e	X	XM	log K_p
10	4	6	30	3.80
50	31	19	95	3.74
200	110	90	450	3.70
500	148	352	1760	3.69
1000	595	405	2025	3.65
			Average log k_p = 3.72	

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	4	5	3
50	27	32	33
200	117	96	121
500	146	129	169
1000	641	538	610

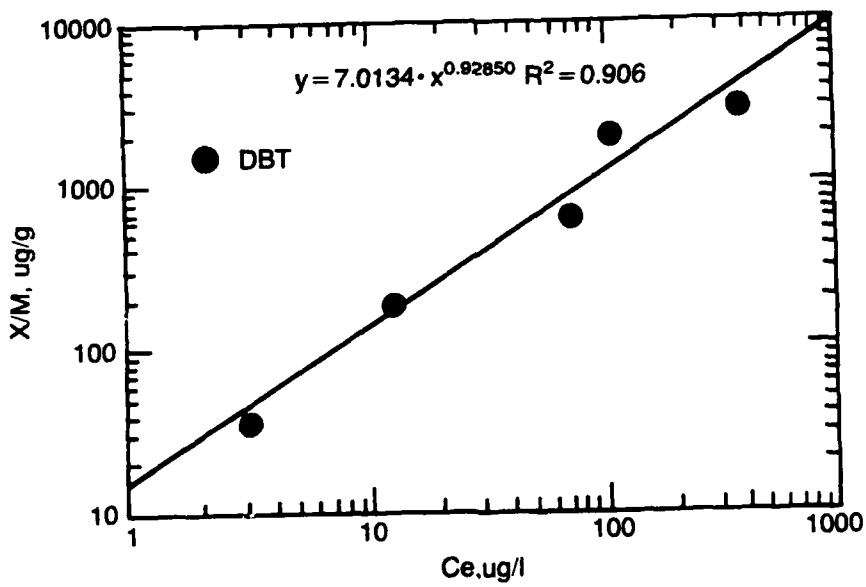

Fig. A.5. Dibutyltin in mixed liquor sludge.

C_o	C_e	X	XM	log K_p
10	6	4	20	3.45
50	33	17	85	3.42
200	80	120	600	3.40
500	296	204	1020	3.37
1000	596	304	1520	3.35
			Average log k_p =	3.40

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	8	5	5
50	34	30	35
200	76	80	84
500	272	285	331
1000	681	682	727

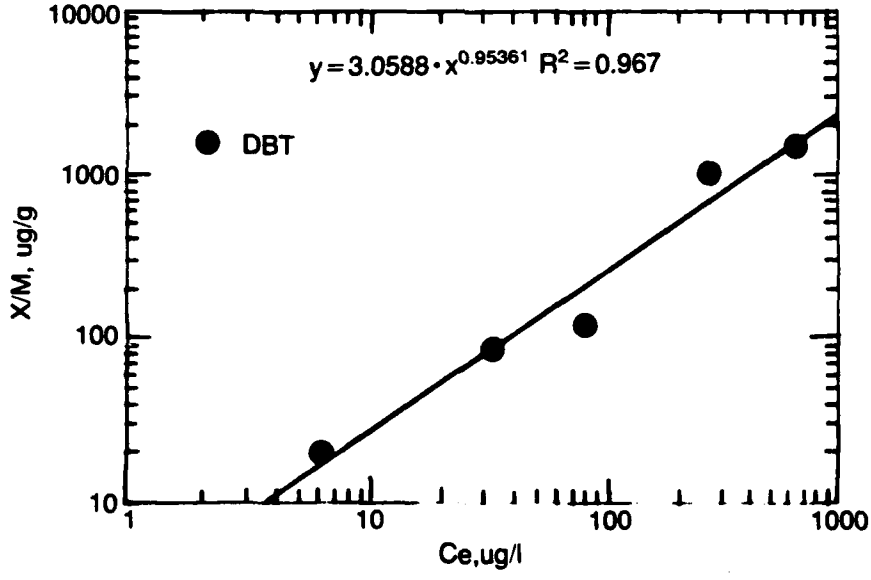

Fig. A.6. Dibutyltin in primary sludge.

C_o	C_e	X	XM	log K_p
10	3	7	35	4.13
50	20	30	150	4.13
200	39	161	805	4.13
500	98	402	2010	4.13
1000	313	687	3435	4.13
			Average log k_p =	4.13

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	3	3	4
50	18	15	26
200	37	46	35
500	103	90	101
1000	313	335	290

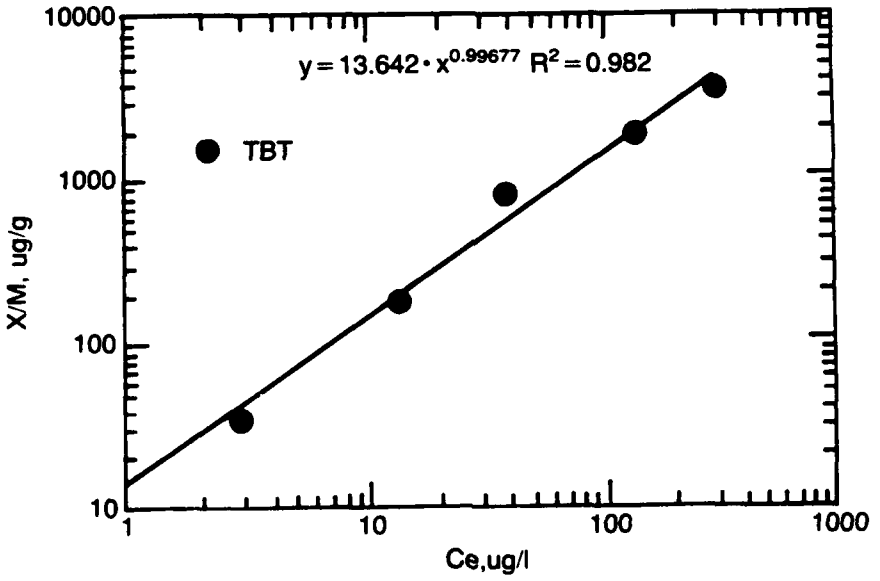

Fig. A.7. Tributyltin in digested sludge.

C_o	C_e	X	XM	log K_p
10	5	5	20	3.84
50	21	29	145	3.89
200	54	146	730	3.92
500	120	380	1900	3.95
1000	487	513	1565	3.99
			Average log k_p =	3.92

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	3	6	6
50	17	27	19
200	60	52	50
500	124	109	126
1000	502	522	437

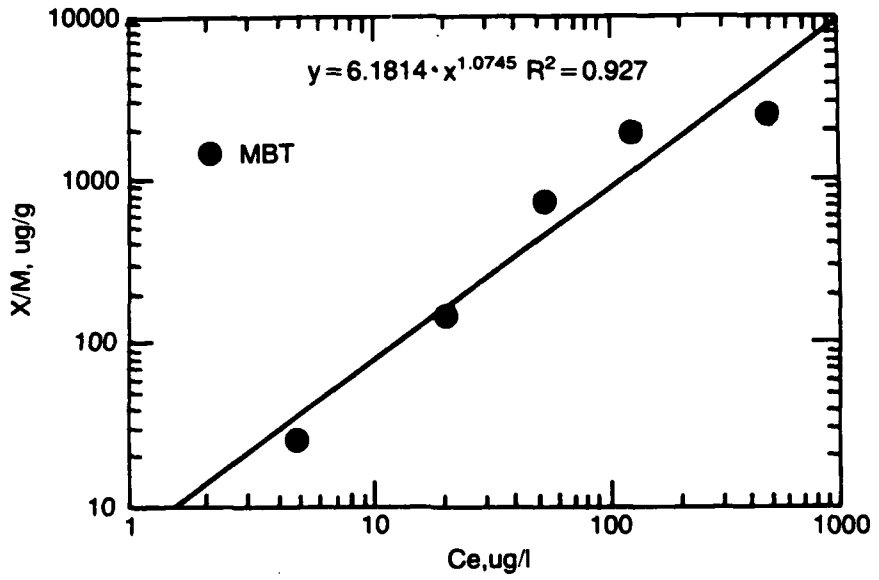

Fig. A.8. Tributyltin in mixed liquor sludge.

C_o	C_e	X	XM	log K_p
10	3	7	35	4.14
50	13	37	185	4.09
200	71	129	645	4.05
500	106	394	1970	4.04
1000	398	602	3010	4.00
			Average log k_p =	3.06

Triplicate Values

	Residual C_e		
C_o	1	2	3
10	3	4	3
50	11	13	16
200	63	65	85
500	93	94	131
1000	279	455	460

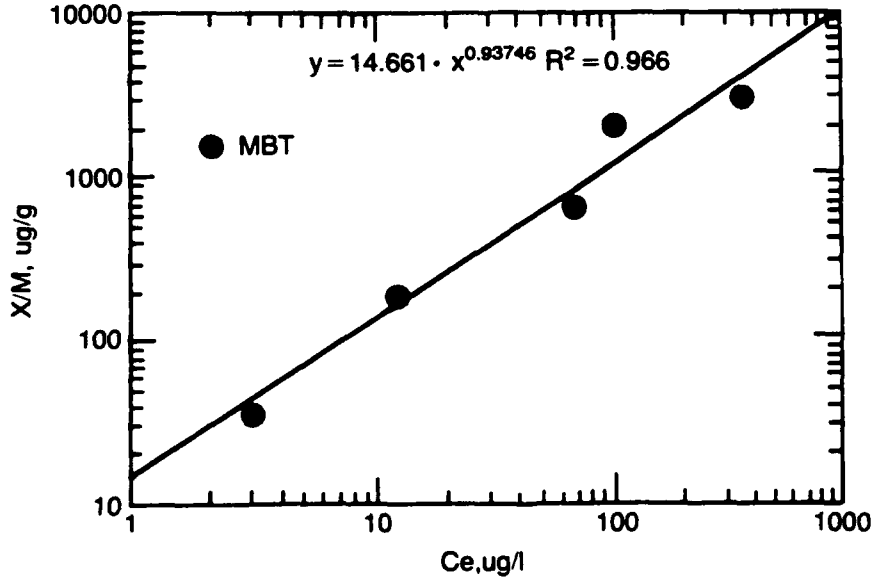

Fig. A.9. Tributyltin in primary sludge.

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